Carbonic Anhydrase Inhibitors: Inhibition of Isozymes I, II, and IX with Triazole-Linked *O*-Glycosides of Benzene Sulfonamides

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Received November 14, 2006

We report the synthesis of a series of benzene sulfonamides containing triazole-*O*-glycoside tails for evaluation as carbonic anhydrase (CA) inhibitors. These glycoconjugates were synthesized by the 1,3-dipolar cycloaddition reaction of 4-azidobenzenesulfonamide with *O*-propynyl glycosides. Compounds were assessed for their ability to inhibit the enzymatic activity of the physiologically dominant isozymes hCA I and II and the tumor-associated isozyme hCA IX (h = human). Against hCA I these compounds were either micromolar or low-nanomolar inhibitors, while against hCA II and IX inhibition in the range of 6.8−53 and 9.7−107 nM, respectively, was observed. The most potent inhibitor against hCA IX was the galactose derivative **8** (K_i = 9.7 nM); this is so far the most potent glycoconjugate inhibitor reported for the tumor-associated hCA IX. These carbohydrate-tethered sulfonamides may prove interesting lead candidates to target tumor-associated CA isozymes, wherein the CA domain is located extracellularly.

Introduction

Carbonic anhydrases[a] (CAs, EC 4.2.1.1) are Zn(II) metalloenzymes that catalyze the reversible hydration of carbon dioxide to give bicarbonate anion and a proton: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$.[1] This reaction is known to regulate a broad range of physiological functions, and clinical modulation of CA activity by inhibitors has proven a reliable treatment for a range of human disease states.[1−4] In humans, 15 different CA isozymes (designated hCA) belonging to the α-CA class are presently known, and many of these isozymes are quite recent discoveries compared with the physiologically abundant and widely distributed isozymes hCA I and II.[1−4] An understanding of the physiological consequences of inhibiting (or activating) specific CA isozymes is developing as the knowledge base regarding CA isozyme enzyme kinetics, tissue distribution, expression levels, and subcellular locations builds. Many of these isozymes have shown promise as druggable targets—with CAs now implicated in areas where human therapies are much needed, for example, in cancer-specific (CA IX, XII)[5−8] and obesity-specific (CA V)[9−11] pathways and in brain function (CA XIV).[12,13] Concomitant with this expanded therapeutic potential is an increased demand for CA isozyme-specific inhibitors—necessary to avoid side effects and to improve therapeutic safety. The classical recognition motif for small molecules to bind the active site of CA is an aromatic sulfonamide moiety ($ArSO_2NH_2$).[1−4,14] The deprotonated sulfonamide ($ArSO_2NH^-$) coordinates to the CA active site Zn^{2+}, and so inhibits the binding of the endogenous substrates (CO_2 and H_2O), thereby reducing the catalytic ability of the enzyme. This CA recognition fragment[15,16] exhibits remarkable reliability in anchoring the inhibitor molecule within the CA active site. The addition of "tail" groups to this zinc-binding functionality has made for an effective means to incorporate into and optimize the chemical and biological properties of sulfonamide CA inhibitors.[14]

Of particular interest in our research is the modulation of CA for cancer applications—either as an anticancer therapy or as a tool for cancer diagnosis and to monitor the effectiveness of treatment. There are two known cancer-associated CA isozymes, CA IX and XII. The genes that encode these isozymes are induced by the hypoxia inducible factor-1 (HIF-1), and elevated levels of CA IX and XII are a marker for a broad spectrum of solid hypoxic tumor types.[8,17,18] The activity of these isozymes contributes to the extracellular acidification (pH_e) of the solid tumor environment, with multiple downstream effects of this reduced pH_e linked to tumor invasion and poor prognosis.[5,17,18] Aromatic sulfonamide compounds (the classical CA inhibitors) have been shown to reverse the effect of tumor acidification, to inhibit the growth of cancer cells, and to suppress tumor invasion mediated by the cancer-associated CAs.[5,17−20] The expression profile of these tumor-associated CAs offers the possibility for CA inhibitors to be developed that can discriminate cancerous cells (overexpression) from healthy cells (minimal expression). Isozymes CA IX and XII also share a global topology that distinguishes them from the physiologically dominant hCA I and II—isozymes IX and XII are transmembrane proteins that orient their CA catalytic domain extracellularly, while isozymes I and II are soluble proteins located in the cytosol. The preparation of aromatic sulfonamides with an impaired ability to diffuse through lipid membranes is therefore one possible means by which to selectively target these isozymes.[21−25] It is well-known that carbohydrates[26,27] have a compromised ability to diffuse across cell membranes, and recently our group has demonstrated that by "click-tailing" sugar moieties to the classical high-affinity aromatic sulfonamide pharmacophore ($ArSO_2NH_2$) we were able to deliver glycoconjugate sulfonamide inhibitors that were potent and selective

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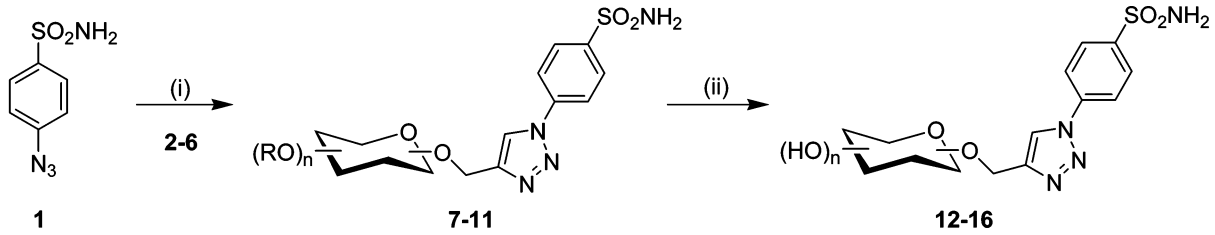
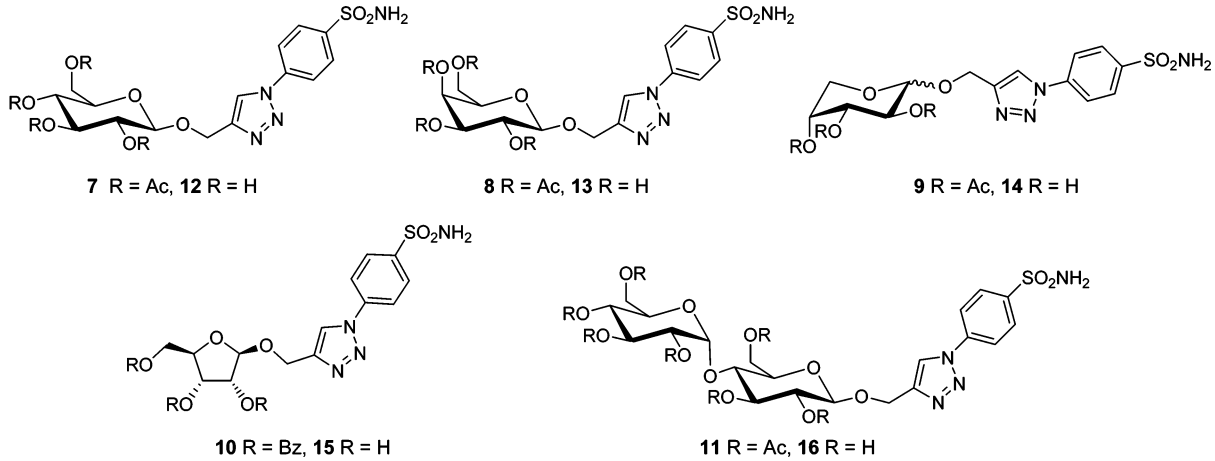
[a] Abbreviations: CA, carbonic anhydrase; 1,3-DCR, 1,3-dipolar cycloaddition reaction; AZA, acetazolamide; MZA, methazolamide; EZA, ethoxzolamide; DCP, dichlorophenamide; BRZ, brinzolamide; IND, Indisulam.

10.1021/jm061320h CCC: $37.00
Published on Web 03/08/2007

Scheme 1[a]

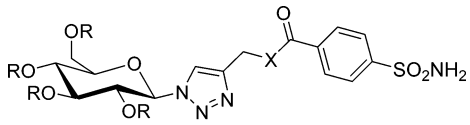


where



[a] Reagents and conditions: (i) azide **1** (0.2−0.5 M), *O*-propynyl glycoside **2**−**6** (1 equiv), $CuSO_4 \cdot 5H_2O$ (0.1−0.2 equiv), sodium ascorbate (0.2−0.4 equiv), 1:1 *t*-BuOH/H_2O, 40 °C, 30 min to 1 h, 77−92%; (R = Ac, Bz) (ii) $NaOCH_3$, CH_3OH, room temperature, 15 min to 2 h, quantitative.

toward the tumor-associated CA isozyme IX in vitro.[24,25] In this earlier study the 1,3-dipolar cycloaddition reaction (1,3-DCR; "click chemistry")[28,29] was employed to generate 1,4-disubstituted 1,2,3-triazole glycoconjugate sulfonamides from azido sugars and alkyne-substituted aromatic sulfonamides. These compounds consisted of a benzene sulfonamide (for CA recognition) linked via an ester or amide moiety to a triazole−sugar tail of the structure type **A**−**D** (here the sugar is derived from *β*-D-glucose).[24,25] The results from this previous study demonstrated that both high affinity and isozyme-selective ligands could be generated using this strategy, and this has encouraged us to continue and extend this work. Herein we explore 1,3-DCR with a reversed "click-tailing" strategy to synthesize a new generation of glycoconjugate sulfonamides by appending *O*-propynyl glycosides onto the 4-azidobenzenesulfonamide CA recognition scaffold (**1**). This library of novel glycoconjugate sulfonamides, now with triazole-*O*-glycoside tail groups, was investigated for their in vitro inhibition of hCA I, II, and tumor-associated hCA IX.



Results and Discussion

Chemistry. A library of 10 benzene sulfonamides (**7**−**16**) containing triazole-tethered *O*-glycoside tails was synthesized by Cu(I)-catalyzed 1,3-DCR of the azido scaffold **1** with a panel of acylated *O*-propynyl glycosides (**2**−**6**) (Figure 1, Scheme 1).[24,25,30] The members of this glycoconjugate library represent a novel structural class with no reported examples of 1,4-disubstituted 1,2,3-triazoles wherein the triazole N-1 substituent was a benzenesulfonamide. Instead of the more chemically robust *N*-triazole-linked carbohydrate as in **A**−**D**,[30,31] the position of the linking 1,2,3-triazole is reversed in this new library, leading to *O*-linked glycosides. The sugar panel encompassed monosaccharide derivatives—pyranoses glucose

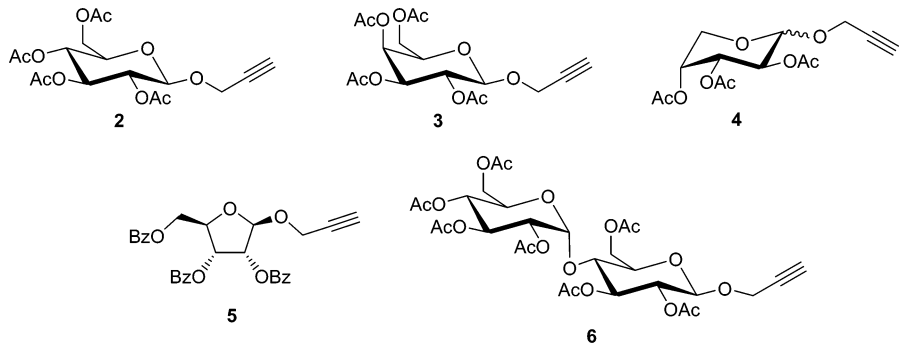
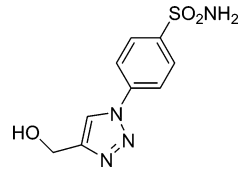


Figure 1. Acylated *O*-propynyl glycoside panel: **2**−**6**.



17

Figure 2. Sulfonamide product (**17**) from potential glycosidase enzyme action on the *O*-glycosides **12**, **13**, and **16**.

(**2**), galactose (**3**), and arabinose (**4**), and a furanose, ribose (**5**)—as well as a disaccharide derivative, maltose (**6**) (Figure 1).

Scaffold **1** was synthesized from commercially available sulfanilamide by diazo transfer under neutral conditions.[32] The peracylated β-*O*-propynyl glycosides **2**, **3**, **5**, and **6** were generated by Lewis acid catalyzed ($BF_3 \cdot Et_2O$ or $SnCl_4$) glycosylation of the β-acetate precursors.[33] The *O*-propynyl arabinoside **4** was prepared as an α/β anomeric mixture using Knoenigs−Knorr conditions (AgOTf) from 2,3,4-tri-*O*-acetyl-β-D-arabinosyl bromide. The *O*-propynyl glycoside panel **2**−**6** were each reacted via Cu(I)-catalyzed 1,3-DCR with the azido arylsulfonamide scaffold **1** to generate glycoconjugate sulfonamides **7**−**11** (Scheme 1). Consistent with our earlier findings, the triazole-forming reaction proceeded smoothly when using a catalyst loading of 10 mol % of the Cu(I) source and 20 mol % of ascorbate.[24,25,30] Reactions were generally complete following 1 h of vigorous stirring (as evidenced by TLC). De-*O*-acylation of **7**−**11** using methanolic sodium methoxide was employed to liberate the corresponding fully deprotected hydroxy sugar analogues **12**−**16** (Scheme 1).

We have included a panel of *O*-glycosides with and without corresponding endogenous glycosidases, such that this second generation of glycoconjugate sulfonamides may have the potential to act as prodrugs for the sulfonamide pharmacophore in the in vivo environment. In recognition that our *O*-glycoside library may exhibit biological activity dependent not only on CA isozyme expression but also on endogenous glycosidase expression, we have also prepared the "clipped" triazole sulfonamide, bearing a 4-CH_2OH alcohol substituent (**17**) (Figure 2). Compound **17** is the expected product from the action of glycosidases on *O*-glycosides **12**, **13**, and **16**. This compound was synthesized similarly to **7**−**11**, by the 1,3-DCR of **1** with propargyl alcohol. There is ample evidence that *O*-glycosides have shown promise both as prodrugs (unmasked in vivo by glycosidases) and for drug targeting (localizing at sites of lectin receptors).[34−36] Several complex *O*-glycosides of natural origin act as potent antitumor agents and antibiotics and include the clinically used anthracyclines and bleomycins. Compelling spectroscopic and crystallographic evidence suggests the glycone moieties of these drugs to be essential structural motifs for the underlying DNA-binding and/or cleavage mechanisms.[36] All sulfonamide compounds (**1**, **7**−**17**) were investigated for their in vitro inhibition of hCA I, II, and IX.

Carbonic Anhydrase Inhibition. hCA I, II, and IX enzyme inhibition data for azide **1**, the 10 new glycoconjugate sulfonamides **7**−**16**, and the alcohol **17** were determined by assaying the CA-catalyzed hydration of CO_2, Table 1.[37] The selectivity ratios for inhibition of isozyme IX compared to those of I and II are also provided in Table 1. Reference data for clinically used CA inhibitors acetazolamide (AZA), methazolamide (MZA), ethoxzolamide (EZA), dichlorophenamide (DCP), and brinzolamide (BRZ) as well as indisulam (IND—an antitumor sulfonamide in phase II clinical trials) have also been included for comparison with the compounds reported in this study.

Table 1. Inhibition and Selectivity Ratio Data for **1**, New Glycoconjugate Sulfonamides **7**−**16**, Alcohol **17**, and Standard Inhibitors against Human Isozymes hCA I, II, and IX

	K_i (nM)[a]			selectivity ratios[b]	
compd	hCA I[c]	hCA II[c]	hCA IX[d]	K_i(hCA I)/(hCA IX)	K_i(hCA II)/(hCA IX)
AZA	900	12	25	36	0.48
MZA	780	14	27	28.9	0.52
EZA	25	8	34	0.74	0.24
BRZ	15	9	42	0.36	0.21
DCP	1200	38	50	24	0.76
IND	31	15	24	1.30	0.63
1	3900	47	105	37.1	0.45
7	1500	46	107	14.0	0.43
8	6.9	6.8	9.7	0.71	0.70
9	5800	9.3	72	80.6	0.13
10	5200	7.6	84	61.9	0.09
11	8.1	7.8	345	0.02	0.02
12	7.0	8.7	101	0.07	0.09
13	4700	53	120	39.2	0.44
14	8.5	6.9	103	0.08	0.07
15	4400	4.2	69	63.8	0.06
16	7.3	48	84	0.09	0.57
17	6200	48	134	46.3	0.36

[a] Errors in the range of ±5−10% of the reported value, from three determinations. [b] The K_i ratios are indicative of isozyme selectivity. [c] Human (cloned) isozymes, by the CO_2 hydration method (refs 37−41). [d] Catalytic domain of human (cloned) isozyme, by the CO_2 hydration method (refs 37−41).

The parent azido scaffold **1** had greatest efficacy against hCA II (K_i of 47 nM), approximately 2-fold weaker inhibition against the tumor-associated hCA IX (105 nM), and 80-fold weaker inhibition against hCA I (3900 nM). The inhibition profile for **1** was weaker at all isozymes compared to that of the standard sulfonamide inhibitors; however, it did have a similar selectivity profile to AZA, MZA, and DCP. Compound **17**, the potential glycosidase product, also had greatest efficacy against hCA II (K_i of 48 nM), 2.8-fold weaker inhibition against hCA IX (134 nM), and 129-fold weaker inhibition against hCA I (6200 nM).

Isozyme hCA I. At hCA I the *O*-glycoside moiety had a variable influence on enzyme inhibition characteristics. The 10 glycoconjugates were investigated both in the acylated form (sugar−OR compounds **7**−**11**) and the free hydroxyl form (sugar−OH compounds **12**−**16**). There were some glycoconjugates (**9**, **10**, **13**, and **15**) that were slightly weaker inhibitors than the parent azido compound **1** with K_i's ranging from 4400−5800 nM, while the acetylated glucose compound **7** was a marginally better inhibitor than **1** (K_i = 1500 nM). The remaining compounds **8**, **11**, **12**, **14**, and **16** exhibited vastly improved inhibition at this isozyme. With K_i's in the low-nanomolar range (6.9−8.5 nM) these compounds were 481- to 565-fold better inhibitors than their azido parent **1**. The inhibition constants for the furanoside derivatives **10** (ribose−OR) and **15** (ribose−OH), and disaccharide derivatives **11** (maltose−OR) and **16** (maltose−OH), were independent of the hydroxyl status of the sugar moiety, with the ribose tail providing micromolar inhibitors and the maltose tail providing low-nanomolar inhibitors. This relationship did not extend to the monosaccharide sugar derivatives (glucose, galactose, and arabinose); here, one of the pair (sugar−OR or sugar−OH) was a nanomolar inhibitor (**12**, **8**, **14**) and the other a micromolar inhibitor (**7**, **13**, **9**). The dependence of inhibition characteristics on the hydroxyl status (sugar−OR or sugar−OH) has important implications for future applications of these compounds, as in vivo they may be exposed to esterases.[43] This structural attribute could in future prove useful for manipulation to suit drug delivery or drug bioavailability demands. Compound **17** was the weakest inhibitor of all sulfonamides evaluated at hCA I.

Isozyme hCA II. At hCA II the parent azido scaffold **1** had a K_i of 47 nM. Seven of the *O*-glycosides (compounds **8**−**12**, **14**, **15**) were potent inhibitors of hCA II (K_i's ranged from 4.2 to 9.3 nM), while compounds **7**, **13**, and **16** had K_i values similar to the azido parent **1** (K_i's ranged from 46−53 nM). Some of the compounds (**8**, **11**, **12**, **14**) exhibited similar inhibition at both hCA I and II, while others (**7**, **9**, **10**, **13**, and **15**) were selective for hCA II. For example, compound **15**, the ribose−OH derivative, was more than 1000-fold selective for hCA II. With regard to selectivity there was one notable exception, the maltose−OH derivative **16**, which was 6.5-fold selective for hCA I and also the only hCA I-selective compound identified in this study. It is apparent that the *O*-glycoside tail groups can strongly influence the selectivity between the physiologically dominant hCA I and II isozymes, demonstrating that the structural diversity of even simple sugars permits noteworthy discrimination of subtle differences in the active site topology of these isozymes. Compound **17** exhibited hCA II inhibition similar to azido scaffold **1** and glycoconjugates **7**, **13**, and **16**.

Isozyme hCA IX. At hCA IX the parent azido scaffold **1** had a K_i of 105 nM, while the alcohol−triazole **17** had a K_i of 134 nM. At this isozyme the *O*-glycoside tails exhibited a relatively clustered pattern of inhibition in contrast to isozymes I and II, with eight of the glycoconjugates exhibiting K_i's in the range of 69−120 nM. The two outliers were the maltose−OAc derivative **11** with a much weaker K_i (345 nM) and the galactose−OAc derivative **8** with a more potent K_i of 9.7 nM (∼11-fold more potent than **1**), respectively. As disaccharide **11** had the bulkiest tail group of those investigated this result may be a qualitative indicator that the steric boundaries within the CA IX active site are being encroached upon. Significantly, compound **8** is more potent than any of the standard sulfonamides—some of which are utilized in physiological studies of this isozyme and none of which are CA IX selective. In terms of SAR the potency of **8** is quite remarkable given that **7** (K_i of 107 nM) and **8** are epimer derivatives with a single structural difference far removed from the sulfonamide CA zinc-binding moiety. It may be drawn from these results that subtle differences in the *O*-glycoside tails can deliver compounds capable of differentially inhibiting CA isozymes. With the exception of **8** the new sulfonamides were weaker inhibitors at CA IX compared to CA II.

Conclusions

The delivery of CA-based cancer therapies or diagnostics will benefit enormously from the development of isozyme-selective inhibitors for the tumor-associated CA isozymes. Selective inhibition among CA isozymes is challenging owing to conservation of active site topology within this enzyme family. With the use of a reversed "click-tailing" strategy, we have appended a structurally diverse panel of *O*-glycosides onto the $ArSO_2NH_2$ CA pharmacophore. This work represents a new class of glycoconjugate CA inhibitors comprising triazole-tethered *O*-glycoside tails. We were able to demonstrate that the glycoconjugates could differentiate the active sites of the widespread isozymes (CA I and II) from the tumor-associated isozyme (CA IX). A very potent hCA IX inhibitor, the galactose−OAc derivative **8** (K_i = 9.7 nM), was identified, demonstrating that sugar tails may underpin a means to generate CA inhibitors for applications in cancer drug development and/or diagnostics. It is also feasible that glycoconjugates could be designed to target endogenous glycosidases in vivo for the purpose of a prodrug therapy. From a synthetic viewpoint the reactions used to generate molecular diversity in carbohydrate-based libraries need to be facile, mild, high yielding, and ideally give a predictable stereochemical outcome. We have shown that the click chemistry 1,3-DCR fulfils these demands. An alternative synthetic route to the library presented herein is the direct glycosylation of the alcohol **17** with 1-*O*-acetate donors. In our hands this is a slower, lower-yielding reaction, which necessitates a more difficult purification when compared to the 1,3-DCR strategy. It is also apparent from both the current and our earlier study that the orientation of the 1,2,3-triazole link between the carbohydrate tail group and the benzene sulfonamide head group strongly influences CA isozyme selectivity profiles, and further manipulation of this structural arrangement will be reported in due course. Finally, an alternative approach to generate glycosidase resistant *S*- and *C*-glycosidic triazole linkages is currently underway within our group.

Experimental Section

Chemistry. Reagents were purchased from the Sigma-Aldrich chemical company and were used without further purification. Solvents were dried and distilled where necessary prior to use or purchased anhydrous from Sigma-Aldrich. Reactions were monitored by TLC using Merck $F60_{254}$ silica plates with visualization of product bands by UV fluorescence (λ = 254 nm) and charring by 10% v/v ethanolic H_2SO_4. Flash chromatography was performed on Merck flash silica gel (0.04−0.06 mm). Melting points were acquired on a Gallenkamp melting point apparatus and are reported as uncorrected. NMR (^1H and ^{13}C $\{^1H\}$) spectra were recorded on a Varian Unity 400 MHz spectrometer at room temperature using DMSO-d_6 solvent unless otherwise stated. Chemical shifts are reported in δ (ppm) from a TMS internal standard (0.0 ppm). Coupling constants (*J*) are reported in hertz. High-resolution electrospray ionization mass spectra were acquired in negative ion mode on an Apex III Bruker Daltonics 4.7T Fourier transform mass spectrometer (FTMS) fitted with an Apollo ESI source.

Synthesis of Sulfonamide Glycosides (7−11). General Procedure A: A mixture of the azide **1** (1.0 equiv) and the appropriate *O*-propynyl glycoside **2**−**6** (1.0 equiv) was suspended in a *tert*-butyl alcohol and water mixture (1:1, 0.2−0.5 M final concentration). A solution of sodium ascorbate (0.2 equiv) in water, followed by a solution $CuSO_4 \cdot 5H_2O$ (0.1 equiv) in water, was added. The bright yellow suspension was stirred vigorously at 40 °C until TLC indicated reaction completion (generally within 2 h). The mixture was evaporated under reduced pressure, and the resulting residue was purified by flash chromatography to yield pure material.

Preparation of Deprotected Sulfonamide Glycosides (12−16). General Procedure B: Compounds **12**−**16** were prepared by the treating the corresponding acylated precursors **7**−**11** (final concentration of ∼0.1−0.2 M; ∼0.5 M concentration for compound **15**) with dry methanolic sodium methoxide (final pH 9−12). Reactions were found to be complete within 30 min (for *O*-acetylates of **12**−**14**, **16**) or 2 h (for *O*-benzoates of **15**) as evidenced by TLC. Neutralization of the solution by Amberlite IR-120 ion-exchange resin, followed by filtration and evaporation of the filtrate to dryness, afforded pure material by NMR.

4-Azidobenzenesulfonamide (1). The preparation of the title compound was adapted from a literature procedure.[32] A slurry of NaN_3 (2.3 g, 34.9 mmol, 3.0 equiv) in distilled H_2O (2 mL) was prepared followed by the addition of *tert*-butyl alcohol (16 mL). Sulfanilamide (2.0 g, 11.6 mmol) was then added followed by the dropwise addition of *tert*-butyl nitrite (16 mL). The deep yellow solution was stirred at room temperature overnight and was found complete by TLC (1:1 EtOAc/hexanes). The crude mixture was diluted with EtOAc (50 mL) and washed with distilled water (50 mL). The aqueous layer was back-extracted with EtOAc (3 × 40 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$), filtered, and evaporated to afford a crude oil. Precipitation from 3:7 ethyl acetate/hexanes (100 mL) and washing with cold hexanes afforded the title compound as a pale yellow crystalline solid (1.7 g, 74%); mp 117−118 °C (decomp; lit mp

119−122 °C);[31] ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26−7.81 (m, 4H, Ph), 7.34 (br s, 2H, SO_2NH_2); ^{13}C {^{1}H} NMR (100 MHz, DMSO-d_6) δ 120.16 (Ph CH), 128.30 (Ph CH), 141.20 (Ph C), 143.60 (Ph C).

4-(4-{[2′,3′,4′,6′-Tetra-*O*-acetyl-*β*-D-glucopyranosyl)oxymethyl}-1-*H*-1,2,3-triazol-1-yl)benzenesulfonamide (7). The title compound was prepared from **2** according to general procedure A and isolated as a white foam following purification by flash chromatography in 3:7 hexanes/EtOAc (175 mg, 0.30 mmol, 77%); R_f 0.63 (100% EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.87 (s, 3H, OAc CH_3), 1.90 (s, 3H, OAc CH_3), 1.96 (s, 3H, OAc CH_3), 1.99 (s, 3H, OAc CH_3), 3.99−4.02 (ddd, $^3J_{5'-4'}$ = 8.8 Hz, $^3J_{5'-6''}$ = 5.6 Hz, $^3J_{5'-6'}$ = 2.4 Hz, 1H, $H_{5'}$), 4.05 (dd, $^2J_{6'-6''}$ = 12.8 Hz, $^3J_{6'-5'}$ = 2.0 Hz, 1H, $H_{6'}$), 4.19 (dd, $^2J_{6''-6'}$ = Hz, 12.4 Hz, $^3J_{6''-5'}$ = 5.2 Hz, 1H, $H_{6''}$), 4.77 (dd, $^3J_{2'-1'}$ = 8.0 Hz, $^3J_{2'-3'}$ = 9.6 Hz, 1H, $H_{2'}$), 4.80 (AB q, $^2J_{AB}$ = 12.4 Hz, 2H, OCH_2), 4.88−4.93 (m, 1H, $H_{4'}$), 4.94 (d, $^3J_{1'-2'}$ = 8.0 Hz, 1H, $H_{1'}$), 5.22−5.27 (m, 1H, $H_{3'}$), 7.50 (br s, 2H, SO_2NH_2), 7.99−8.11 (m, 4H, Ph), 8.87 (s, 1H, triazole CH); ^{13}C {^{1}H} NMR (100 MHz, DMSO-d_6) δ 20.94 (OAc CH_3), 21.01 (OAc CH_3), 21.06 (OAc CH_3), 21.18 (OAc CH_3), 62.24 ($C_{6'}$), 62.36 (OCH_2), 68.79 ($C_{4'}$), 71.36 ($C_{5'}$), 71.53 ($C_{2'}$), 72.73 ($C_{3'}$), 99.21 ($C_{1'}$), 121.02 (Ph CH), 123.72 (triazole CH), 128.20 (Ph CH), 139.22 (Ph C), 144.58 (triazole C or Ph C), 145.02 (triazole C or Ph C), 169.69 (C═O), 169.53 (C═O), 170.21 (C═O), 170.75 (C═O); HRMS (ESI) calcd for $C_{23}H_{27}N_4O_{12}S^-$: 583.135392. Found: 583.134630. Anal. ($C_{23}H_{28}N_4O_{12}S\cdot H_2O$) H, N, S; C calcd, 45.84; found, 46.54.

4-(4-{[2′,3′,4′,6′-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-oxymethyl}-1-*H*-1,2,3-triazol-1-yl)benzenesulfonamide (8). The title compound was prepared from **3** according to general procedure A and isolated as a pale yellow foam following purification by flash chromatography in 3:7 hexanes/EtOAc (263 mg, 0.45 mmol, 87%); R_f 0.23 (1:4 hexanes/EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.87 (s, 3H, OAc CH_3), 1.91 (s, 3H, OAc CH_3), 1.98 (s, 3H, OAc CH_3), 2.09 (s, 3H, OAc CH_3), 4.01−4.10 (m, 2H, H_6, $H_{6'}$), 4.20−4.23 (m, 1H, H_5), 4.80 (AB q, $^2J_{AB}$ = 12.4 Hz, 2H, OCH_2), 4.84 (d, $^3J_{1'-2'}$ = 8.0 Hz, 1H, $H_{1'}$), 4.93 (dd, $^3J_{2'-3'}$ = 10.0 Hz, $^3J_{2'-1'}$ = 8.0 Hz, 1H, $H_{2'}$), 5.14 (dd, $^3J_{3'-2'}$ = 10.0 Hz, $^3J_{3'-4'}$ = 3.6 Hz, 1H, $H_{3'}$), 5.23−4.25 (m, 1H, $H_{4'}$), 7.49 (br s, 2H, SO_2NH_2), 7.98−8.11 (m, 4H, Ph), 8.88 (s, 1H, triazole CH); ^{13}C {^{1}H} NMR: δ 21.00 (OAc CH_3), 21.07 (OAc CH_3), 21.11 (OAc CH_3), 21.18 (OAc CH_3), 61.96 (OCH_2), 62.18 ($C_{6'}$), 67.99 ($C_{4'}$), 69.26 ($C_{3'}$), 70.69 ($C_{2'}$), 70.88 ($C_{5'}$), 99.71 ($C_{1'}$), 121.00 (Ph CH), 123.65 (triazole CH), 128.22 (Ph C), 139.20 (Ph C), 144.60 (triazole C or Ph C), 145.09 (triazole C or Ph C), 169.79 (OAc C═O), 170.15 (OAc C═O), 170.60 (OAc C═O), 170.62 (OAc C═O). HRMS (ESI) calcd for $C_{23}H_{27}N_4O_{12}S^-$: 582.135392. Found: 583.134423. Anal. ($C_{23}H_{28}N_4O_{12}S$) C, H, N, S.

4-(4-{[2′,3′,4′-Tri-*O*-acetyl-α/*β*-D-arabinopyranosyl)oxymethyl}-1-*H*-1,2,3-triazol-1-yl)benzenesulfonamide (9). The title compound was prepared from **4** according to general procedure A and isolated as a white foam following flash chromatography in 1:4 hexanes/EtOAc (as a 2:1 α/*β* mixture by ^{1}H NMR) (145 mg, 0.28 mmol, 89%); R_f 0.30 (1:4 hexanes/EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.90 (s, 3H, αOAc CH_3), 1.91 (s, 6H, α and *β*OAc CH_3), 1.93 (s, 3H, *β*OAc CH_3), 2.07 (s, 3H, αOAc CH_3), 2.08 (s, 3H, *β*OAc CH_3), 3.67 (dd, $^2J_{5'-5''}$ = 13.2 Hz, $^3J_{5'-4'}$ = 2.0 Hz, 1H, *β*$H_{5'}$), 3.83 (dd, $^2J_{5'-5''}$ = 13.2 Hz, $^3J_{5'-4'}$ = 2.0 Hz, 1H, α$H_{5'}$), 3.90 (dd, $^2J_{5''-5'}$ = 13.2 Hz, $^3J_{5''-4'}$ = 2.4 Hz, α$H_{5''}$), 4.02 (dd, $^2J_{5''-5'}$ = 13.2 Hz, $^3J_{5''-4'}$ = 2.4 Hz, 1H, *β*$H_{5''}$), 4.72 (d, $^3J_{1'-2'}$ = 8.0 Hz, 1H, α$H_{1'}$), 4.73 (d, $^3J_{1'-2'}$ = 2.8 Hz, 1H, *β*$H_{1'}$), 4.74 (AB q, $^2J_{AB}$ = 12.8 Hz, 2H, *β*OCH_2), 4.78 (AB q, $^2J_{AB}$ = 12.8 Hz, 2H, αOCH_2), 4.93 (dd, $^3J_{2'-3'}$ = 10.0 Hz, $^3J_{2'-1'}$ = 8.0 Hz, 1H, α$H_{2'}$), 4.97 (dd, $^3J_{2'-3'}$ = 10.4 Hz, $^3J_{2'-1'}$ = 3.2 Hz, 1H, *β*$H_{2'}$), 5.08 (dd, $^3J_{3'-2'}$ = 9.6 Hz, $^3J_{3'-4'}$ = 3.2 Hz, 1H, *β*$H_{3'}$), 5.12−5.14 (m, 1H, α$H_{4'}$), 5.18 (dd, $^3J_{3'-2'}$ = 11.2 Hz, $^3J_{3'-4'}$ = 3.6 Hz, 1H, α$H_{3'}$), 5.23−5.25 (m, 1H, *β*$H_{4'}$), 7.49 (br s, 2H, SO_2NH_2), 7.98−8.11 (m, 4H, Ph CH), 8.89 (s, 1H, α triazole CH), 8.96 (s, 1H, *β* triazole CH); ^{13}C {1H} NMR (100 MHz, DMSO-d_6) δ 21.07 (OAc CH_3), 21.13 (OAc CH_3), 21.37 (OAc CH_3), 61.84 (OCH_2), 63.99 ($C_{5'}$), 68.60 ($C_{4'}$), 69.51 ($C_{2'}$), 70.63 ($C_{3'}$), 99.87 ($C_{1'}$), 120.99 (Ph CH), 123.56 (triazole CH), 128.21 (Ph CH), 139.21 (Ph C), 145.56 (triazole C or Ph C), 145.13 (triazole C or Ph C), 169.82 (C═O), 170.23 (C═O), 170.51 (C═O). HRMS (ESI) calcd for $C_{20}H_{23}N_4O_{10}S^-$: 511.114249. Found: 511.113207. Anal. ($C_{20}H_{24}N_4O_{10}S\cdot 0.5\ H_2O$) C, H, N.

4-(4-{[2′,3′,5′-Tri-*O*-benzoyl-*β*-D-ribofuranosyl)oxymethyl}-1-*H*-1,2,3-triazol-1-yl)benzenesulfonamide (10). The title compound was prepared from **5** according to general procedure A and isolated as a pale yellow foam following flash chromatography (1:1 EtOAc/hexanes; 675 mg, 0.97 mmol, 91%); R_f 0.53 (2:3 hexanes/EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.51 (dd, $^2J_{5'-5''}$ = 12.0 Hz, $^3J_{5-4}$ = 5.2 Hz, 1H, $H_{5'}$), 4.66 (dd, $^2J_{5''-5'}$ = 12.0 Hz, $^3J_{5''-4'}$ = 4.0 Hz, 1H, $H_{5''}$), 4.78−4.82 (m, 1H, $H_{4'}$), 4.81 (AB q, $^2J_{AB}$ = 12.4 Hz, 2H, OCH_2), 5.52 (s, 1H, H_1), 5.60 (d, $^3J_{2'-3'}$ = 4.8 Hz, 1H, H_2), 5.77 (d, $^3J_{3'-4'}$ = 6.8 Hz, $^3J_{3'-2'}$ = 4.8 Hz, 1H, H_3), 7.34−8.07 (m, 21 H, Ph CH and SO_2NH_2), 8.84 (s, 1H, triazole CH); ^{13}C {^{1}H} NMR (100 MHz, DMSO-d_6) δ 61.05 (OCH_2), 65.06 ($C_{5'}$), 72.59 ($C_{3'}$), 75.68 ($C_{2'}$), 79.17 ($C_{4'}$), 104.67 ($C_{1'}$), 120.93 (Ph CH), 123.35 (triazole CH), 128.14, 129.26, 129.31, 129.35, 129.55, 129.87, 129.90, 130.04 (Ph CH), 134.06, 134.47, 134.62, 139.21, 144.48 (Ph C), 145.21 (triazole C), 166.11 (C═O), 165.49 (C═O), 165.32 (C═O). HRMS (ESI) calcd for $C_{35}H_{29}N_4O_{10}S^-$: 699.17539. Found: 699.174859. Anal. ($C_{35}H_{30}N_4O_{10}S\cdot H_2O$) C, H, N, S.

4-(4-{[Hepta-*O*-acetyl-*β*-D-maltopyranosyl)oxymethyl}-1-*H*-1,2,3-triazol-1-yl)benzenesulfonamide (11). The title compound was prepared from **6** according to general procedure A and isolated as a pale yellow foam after flash chromatography in 1:1 EtOAc/hexanes (166 mg, 0.19 mmol, 86%); R_f 0.22 (1:1 EtOAc/hexanes); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.86 (s, 3H, OAc CH_3), 1.90 (s, 3H, OAc CH_3), 1.92 (s, 3H, OAc CH_3), 1.94 (s, 3H, OAc CH_3), 1.96 (s, 3H, OAc CH_3), 1.99 (s, 3H, OAc CH_3), 2.05 (s, 3H, OAc CH_3), 3.93−4.03 (m, 4H, Glc*β* $H_{4'}$, Glcα $H_{5'}$, Glc*β* $H_{5'}$, Glcα or Glc*β* $H_{6''}$), 4.14 (dd, $^2J_{6'-6''}$ = 12.0 Hz, $^3J_{6'-5'}$ = 4.4 Hz, 1H, Glcα or Glc*β* $H_{6'}$), 4.18 (dd, $^2J_{6'-6''}$ = 12.4 Hz, $^3J_{6'-5'}$ = 4.8 Hz, 1H, Glcα or Glc*β* $H_{6''}$), 4.39 (dd, $^2J_{6''-6'}$ = 12.0 Hz, $^3J_{6''-5'}$ = 2.4 Hz, 1H, Glcα or Glc*β* $H_{6''}$), 4.67 (dd, $^3J_{2'-3'}$ = 9.6 Hz, $^3J_{2'-1'}$ = 8.0 Hz, 1H, Glc*β* $H_{2'}$), 4.78 (AB q, $^2J_{AB}$ = 12.8 Hz, 2H, OCH_2), 4.84 (dd, $^3J_{2'-3'}$ = 9.6 Hz, $^3J_{2'-1'}$ = 3.6 Hz, 1H, Glcα $H_{2'}$), 4.91 (d, $^3J_{1'-2'}$ = 8.0 Hz, 1H, Glc*β* $H_{1'}$), 4.93−4.98 (m, 1H, Glcα $H_{4'}$), 5.17−5.22 (m, 1H, Glcα $H_{3'}$), 5.25 (d, $^3J_{1'-2'}$ = 3.6 Hz, 1H, Glcα $H_{1'}$), 5.25−5.30 (m, 1H, Glc*β* $H_{3'}$), 7.50 (br s, 2H, SO_2NH_2), 7.99−8.11 (m, 4H, Ph CH), 8.85 (s, 1H, triazole CH); ^{13}C {^{1}H} NMR (100 MHz, DMSO-d_6) δ 21.95 (OAc CH_3), 21.02 (2 × OAc CH_3), 21.11 (OAc CH_3), 21.21 (OAc CH_3), 21.26 (OAc CH_3), 21.44 (OAc CH_3), 60.43 (Glc $C_{6'}$), 62.07 (Glc $C_{6'}$), 63.54 (OCH_2), 68.42 (Glc $C_{4'}$), 68.68 (Glc $C_{4'}$), 69.53 (Glc $C_{3'}$), 70.11 (Glc $C_{3'}$), 72.02 (Glc $C_{2'}$), 72.20 (Glc $C_{2'}$), 74.30 (Glc $C_{5'}$), 74.99 (Glc $C_{5'}$), 96.05 (Glc*β* $H_{1'}$), 98.75 (Glcα $H_{1'}$), 121.03 (Ph CH), 123.73 (triazole CH), 128.19 (Ph CH), 139.22 (Ph C), 144.57 (triazole C or Ph C), 144.99 (triazole C or Ph C), 169.83 (OAc C═O), 169.88 (OAc C═O), 170.27 (OAc C═O), 170.54 (OAc C═O), 170.69 (OAc C═O), 170.85 (OAc C═O). HRMS (ESI) calcd for $C_{35}H_{43}N_4O_{20}S^-$: 871.219684. Found: 871.220422. Anal. ($C_{35}H_{44}N_4O_{20}S\cdot H_2O$) C, H, N, S.

4-(4-{[*β*-D-Glucopyranosyl)oxymethyl}-1-*H*-1,2,3-triazol-1-yl)benzenesulfonamide (12). The title compound was prepared from **7** according to general procedure B and isolated as a white solid (107 mg, 0.26 mmol, ∼100%); R_f 0.12 (1:9 H_2O/CH_3CN); mp 244−246 °C (decomp); ^{1}H NMR (400 MHz, 10% DMSO-d_6 in D_2O) δ 3.16−3.20 (m, 1H, $H_{2'}$), 3.23−3.28 (m, 1H, $H_{4'}$), 3.33−3.38 (m, 2H, $H_{3'}$, $H_{5'}$), 3.58 (dd, $^2J_{6'-6''}$ = 12.8 Hz, $^3J_{6'-5'}$ = 5.6 Hz, 1H, $H_{6'}$), 3.78 (dd, $^2J_{6''-6'}$ = 12.4 Hz, $^3J_{6''-5'}$ = 2.0 Hz, 1H, $H_{6''}$), 4.47 (d, $^3J_{1'-2'}$ = 8.0 Hz, 1H, $H_{1'}$), 4.89 (AB q, $^2J_{AB}$ = 12.8 Hz, 2H, OCH_2), 7.86−7.99 (m, 4H, Ph), 8.49 (s, 1H, triazole CH); ^{13}C {^{1}H} NMR (100 MHz, 10% DMSO-d_6 in D_2O) δ 60.96 ($C_{6'}$), 62.09 (OCH_2), 69.84 ($C_{4'}$), 73.23 ($C_{3'}$), 75.92 ($C_{2'}$), 76.21 ($C_{5'}$), 101.84 ($C_{1'}$), 121.75 (Ph CH), 124.00 (triazole CH), 128.08 (Ph CH), 139.54 (Ph C), 142.24 (triazole C or Ph C), 144.922 (triazole C or Ph C). HRMS (ESI) calcd for $C_{15}H_{19}N_4O_8S^-$: 415.02908. Found: 415.093029.

4-(4-{[*β*-D-Galactopyranosyl)oxymethyl}-1-*H*-1,2,3-triazol-1-yl)benzenesulfonamide (13). The title compound was prepared

from **8** according to general procedure B and isolated as a white solid (150 mg, 0.36 mmol, ~100%); R_f 0.13 (1:9 H_2O/CH_3CN); mp 241−244 °C (decomp); ^{1}H NMR (400 MHz, D_2O) δ 3.42 (dd, $^3J_{2'-3'} = 9.6$ Hz, $^3J_{2'-1'} = 7.6$ Hz, 1H, $H_{2'}$), 3.50 (dd, $^3J_{3'-2'} = 10.0$ Hz, $^3J_{3'-4'} = 3.6$ Hz, 1H, $H_{3'}$), 3.56−3.69 (m, 3H, $H_{5'}$, $H_{6'}$, $H_{6''}$), 3.78−3.79 (m, 1H, $H_{4'}$), 4.41 (d, $^3J_{1'-2'} = 7.6$ Hz, 1H, $H_{1'}$), 4.91 (AB q, $^2J_{AB} = 12.4$ Hz, 2H, OCH_2), 4.89−8.01 (m, 4H, Ph), 8.53 (s, 1H, triazole CH); ^{13}C {^{1}H} NMR (100 MHz, DMSO-d_6) δ 61.19 ($C_{6'}$), 62.01 (OCH_2), 68.79 ($C_{4'}$), 71.10 ($C_{3'}$), 73.89 ($C_{2'}$), 76.0 ($C_{5'}$), 103.59 ($C_{1'}$), 120.92 (Ph CH), 123.50 (triazole CH), 128.25 (Ph CH), 139.29 (Ph C), 144.44 (triazole C or Ph C), 146.211 (triazole C or Ph C). HRMS (ESI) calcd for $C_{15}H_{19}N_4O_8S^-$: 415.092908. Found: 415.093072.

4-(4-{[α/*β*-D-Arabinopyranosyl)oxymethyl}-1-*H*-1,2,3-triazol-1-yl)benzensulfonamide (14). The title compound was prepared from **9** according to general procedure B and isolated as a white solid (as a 2:1 α/β mixture by ^{1}H NMR) (75 mg, 0.19 mmol, ~100%); R_f 0.23 (1:9 CH_3OH/EtOAc); ^{1}H NMR (400 MHz, 2% D_2O in DMSO-d_6) δ 3.31−3.47 (m, 4H, $\alpha H_{2'}$, $\alpha H_{5'}$, $\alpha H_{5''}$, $\beta H_{5'}$ and $\beta H_{5''}$), 3.56−3.58 (m, 1H, $\beta H_{2'}$), 3.60−3.62 (m, 1H, $\alpha H_{4'}$), 3.67−3.73 (m, 4H, $\alpha H_{3'}$, $\alpha H_{4'}$, $\beta H_{4'}$), 3.71 (dd, $^3J_{3'-2'} = 12.0$ Hz, $^3J_{3'-4'} = 3.6$ Hz, 1H, $\beta H_{3'}$), 4.23 (d, $^3J_{1'-2'} = 6.0$ Hz, 1H, $\alpha H_{1'}$), 4.66 (AB q, $^2J_{AB} = 12.0$ Hz, 1H, βOCH_2), 4.75 (AB q, $^2J_{AB} = 12.8$ Hz, 2H, αOCH_2), 4.80 (d, $^3J_{1'-2'} = 2.4$ Hz, 1H, $\beta H_{1'}$), 7.98−8.10 (m, 4H, Ph CH), 8.87 (s, 1H, α triazole CH), 8.89 (s, 1H, β triazole CH); ^{13}C {1H} NMR (100 MHz, DMSO-d_6) δ 60.73 (β OCH_2), 61.44 (α OCH_2), 63.95, 65.87, 67.99, 68.79, 69.06, 69.49, 71.03, 72.95, (αC_2, αC_3, $\alpha C_{4'}$, $\alpha C_{5'}$ and, $\beta C_{2'}$, $\beta C_{3'}$, $\beta C_{4'}$, $\beta C_{5'}$), 99.60 ($\beta C_{1'}$), 103.05 ($\alpha C_{1'}$), 120.93 (βPh CH), 120.65 (αPh CH), 123.18 (α triazole CH), 123.32 (β triazole Ph CH), 128.24 (Ph CH), 139.30 (Ph C), 144.36 (Ph C), 146.05 (α triazole C), 146.15 (β triazole C). HRMS (ESI) calcd for $C_{14}H_{17}N_4O_7S^-$: 385.082343. Found: 385.082537.

4-(4-{[*β*-D-Ribofuranosyl)oxymethyl}-1-*H*-1,2,3-triazol-1-yl)-benzenesulfonamide (15). The title compound was prepared from **10** according to general procedure B and isolated as a pale yellow solid following flash chromatography (1:1 EtOAc/hexanes then 1:9; 112 mg, 0.29 mmol, ~100%); R_f 0.12 (1:9 EtOAc/CH_3OH); mp 146−147 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.37 (dd, $^2J_{5'-5''} = 12.0$ Hz, $^3J_{5'-4'} = 6.4$ Hz, 1H, $H_{5'}$), 3.54 (dd, $^2J_{5''-5'} = 11.6$ Hz, $^3J_{5''-4'} = 3.72$, 1H, $H_{5''}$), 3.73 (d, $^3J_{2'-3'} =$ Hz, 1H, $H_{2'}$), 3.79 (ddd, $^3J_{4'-3'} = 10.4$ Hz, $^3J_{4'-5'} = 6.4$ Hz, $^3J_{4'-5''} = 4.0$ Hz, 1H, $H_{4'}$), 3.87 (dd, 1H, $H_{3'}$), 4.67 (AB q, $^2J_{AB} = 12.4$ Hz, 2H, CH_2O), 4.88 (s, 1H, $H_{1'}$), 7.97−8.11 (m, 4H, Ph), 8.85 (s, 1H, triazole CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 60.10 ($C_{5'}$), 63.62 (CH_2O), 71.43 ($C_{3'}$), 74.93 ($C_{2'}$), 84.43 ($C_{4'}$), 106.83 ($C_{1'}$), 120.98 (Ph CH), 123.21 (triazole CH), 128.20 (Ph CH), 139.31 (Ph C), 144.38 (triazole C or Ph C), 146.04 (triazole C or Ph C). HRMS (ESI) calcd for $C_{14}H_{18}N_4O_7SNa^+$: 409.078838. Found: 409.078059.

4-(4-{[*β*-D-Maltopyranosyl)oxymethyl}-1-*H*-1,2,3-triazol-1-yl)benzenesulfonamide (16). The title compound was prepared from **11** according to general procedure B and isolated as an off-white solid (99 mg, 0.17 mmol, ~100%); R_f 0.09 (1:9 H_2O/CH_3CN); mp 192−196 °C (decomp). ^{1}H NMR (400 MHz, D_2O) δ 3.22 (dd, $^3J_{2'-3'} = 9.2$ Hz, $^3J_{2'-1'} = 8.0$ Hz, 1H, Glcβ $H_{2'}$), 3.25−3.30 (m, 1H, Glcβ $H_{4'}$), 3.43 (dd, $^3J_{2'-3'} = 10.0$ Hz, $^3J_{2'-1'} = 4.0$ Hz, Glcα $H_{1'}$), 3.49−3.66 (m, 7H, Glcα $H_{3'}$, Glcα H_4, Glcα H_5, Glcα H_6 and Glcβ H_3, Glcβ H_5, Glcβ H_6), 3.71 (dd, $^2J_{6''-6'} = 12.0$ Hz, $^3J_{6''-5'} = 2.0$ Hz, 1H, Glcα $H_{6''}$ or Glcβ $H_{6''}$), 3.80 (dd, $^2J_{6''-6'} = 12.4$ Hz, $^3J_{6''-5'} = 1.6$ Hz, 1H, Glcα $H_{6''}$ or Glcβ $H_{6''}$), 4.49 (d, $^3J_{1'-2'} = 8.0$ Hz, 1H, 1H, Glcβ $H_{1'}$), 4.90 (AB q, $^2J_{AB} = 12.4$ Hz, 2H, OCH_2), 5.26 (d, $^3J_{1'-2'} = 3.6$ Hz, 1H, Glcα $H_{1'}$), 7.87−7.99 (m, 4H, Ph), 8.49 (s, 1H, triazole CH); ^{13}C {^{1}H} NMR (100 MHz, D_2O) δ 60.23 (OCH_2), 60.87, 62.10, 69.48, 71.79, 72.85, 72.97, 73.07, 74.78, 76.33, 76.81 (Glcα $C_{2'}$, Glcα $C_{3'}$, Glcα $C_{4'}$, Glcα $C_{5'}$, Glcα $C_{6'}$ and Glcβ $C_{2'}$, Glcβ $C_{3'}$, Glcβ $C_{4'}$, Glcβ $C_{5'}$, Glcβ $C_{6'}$), 99.70 (Glcα $C_{1'}$), 101.59 (Glcβ $C_{1'}$), 121.75 (Ph CH), 124.06 (triazole CH), 128.02 (Ph CH), 139.58 (Ph C), 141.93 (triazole C or Ph C), 144.74 (triazole C or Ph C). HRMS (ESI) calcd for $C_{21}H_{29}N_4O_{13}S^-$: 577.145731. Found: 577.145862.

CA Inhibition Assay. An Applied Photophysics stopped-flow instrument has been used for assaying the CA-catalyzed CO_2 hydration activity.[37] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5) as buffer, 0.1 M Na_2SO_4 (for maintaining constant the ionic strength), at 25 °C, following the CA-catalyzed CO_2 hydration reaction for a period of 10−100 s (the uncatalyzed reaction needs around 60−100 s in the assay conditions, whereas the catalyzed ones are of around 6−10 s). The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of kinetic parameters. For each inhibitor, tested in the concentration range between 0.01 nM and 100 μM, at least six traces of the initial 5−10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (1 mM) were prepared in distilled−deionized water with 10−20% (v/v) DMSO (which is not inhibitory at these concentrations), and dilutions up to 0.01 nM were done thereafter with distilled−deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E−I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3. The curve-fitting algorithm allowed us to obtain the IC_{50} values, working at the lowest concentration of substrate of 1.7 mM, from which K_i values were calculated by using the Cheng−Prusoff equation.[38−42] The catalytic activity (in the absence of inhibitors) of these enzymes was calculated from Lineweaver−Burk plots, as reported earlier, and represent the mean from at least three different determinations.[38−42] Enzyme concentrations in the assay system were 9.2 nM for hCA I, 7.3 nM for hCA II, and 8.5 nM for hCA IX. Enzymes used here were recombinant ones, prepared and purified as described earlier.[38−42,44]

Acknowledgment. This work was financed in part by the Australian Research Council (Grant Nos. F00103312 and DP0343419), the Eskitis Institute for Cell and Molecular Therapies and the School of Science (Griffith University, S.-A.P.), and an EU Grant of the sixth framework programme (EUROXY project) (University of Florence, C.T.S.). We thank Hoan The Vu for carrying out accurate mass measurements.

Supporting Information Available: Elemental analysis data and ^{1}H NMR spectra for compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM061320H